

Original
articleDetection of *Chlamydia trachomatis* in vaginal specimens from female commercial sex workers using a new improved enzyme immunoassay

Masatoshi Tanaka, Hiroshi Nakayama, Hiroshi Yoshida, Koichi Takahashi, Tatsuo Nagafuji, Toshikatsu Hagiwara, Joichi Kumazawa

Objective: To evaluate the performance of a new improved enzyme immunoassay (EIA) kit for the detection of *Chlamydia trachomatis* in vaginal swab and endocervical swab specimens from female commercial sex workers, in comparison with a conventional EIA test and a polymerase chain reaction (PCR) assay.

Methods: A high risk group of 163 female commercial sex workers who visited a sexually transmitted disease (STD) clinic in order to undergo screening for major STDs, including chlamydial infection, were enrolled. A total of four swab specimens, including two vaginal and two endocervical specimens, were collected from each woman by a clinician. To identify *C trachomatis*, a new improved EIA kit (IDEIA PCE), a conventional EIA kit (IDEIA), and PCR assay (Amplicor) were used. Discrepancies in the results were resolved using supplementary PCR assay. A female patient was considered to be infected with *C trachomatis* if the IDEIA PCE test and PCR test for both sample sites (endocervical and vaginal) gave positive results. Following resolution of these discrepancies, relative sensitivity and specificity, confidence intervals, and predictive values for each type of specimen by each assay were calculated.

Results: Of the 163 women tested, 35 (21.5%) were shown to be infected with *C trachomatis*. The relative sensitivities in vaginal swab specimens were 88.8%, 68.6%, and 91.4% using IDEIA PCE, IDEIA, and PCR, respectively. The relative specificities in vaginal swab specimens were 99.2%, 99.2%, and 100%, respectively. The relative sensitivities in endocervical swab specimens were 85.7%, 77.1%, and 91.4% with IDEIA PCE, IDEIA, and PCR, respectively. The relative specificities in endocervical swab specimens were all 100%.

Conclusions: The results obtained in this study suggest that the sensitivity and specificity of IDEIA PCE test on vaginal swab and endocervical swab specimens were similar to those of PCR assay on the two types of specimen. It is concluded that IDEIA PCE test on vaginal swab specimens is an acceptable, sensitive, and less invasive approach for the detection of *C trachomatis* in commercial sex workers with a high prevalence of *C trachomatis* infection.

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Keywords: enzyme immunoassay; *Chlamydia trachomatis*; vaginal specimens; endocervical specimens; commercial sex workers

**Department of
Urology, Faculty of
Medicine, Kyushu
University, Japan**

M Tanaka
K Takahashi
T Nagafuji
J Kumazawa

**Nakayama Urologic
Clinic, Fukuoka, Japan**

H Nakayama

**Department of
Virology, National
Institute of Infectious
Diseases, Tokyo, Japan**

H Yoshida
T Hagiwara

Correspondence to:
Masatoshi Tanaka, MD,
Department of Urology,
Faculty of Medicine, Kyushu
University, 3–1–1 Maidashi,
Higashi-ku, Fukuoka 812,
Japan.

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Introduction

Chlamydia trachomatis infection is the most common bacterial sexually transmitted disease (STD) in the developed countries.¹ Our previous study has demonstrated that *C trachomatis* infection is also the most prevalent STD in Fukuoka City, Japan, and that commercial sex workers play an important role as a reservoir in the spread of *C trachomatis* infection.²

Recently, to detect *C trachomatis* in the clinical samples, nucleic acid amplification techniques such as polymerase chain reaction (PCR) and ligase chain reaction (LCR) have been developed. These DNA amplification methods are more sensitive than cultural recovery or antigen detection tests, such as enzyme immunoassay (EIA).^{3–6} However, despite the advent of DNA amplification technology, the EIA test is still widely used for the diagnosis of *C trachomatis* in Japan.

Currently, DNA amplification testing of vaginal specimens obtained by clinicians or patients themselves has been reported to have the sensitivity as that of endocervical specimens.^{7–10} Vaginal swab specimens seem to

be more suitable for the screening of *C trachomatis* infection as a means of less invasive sampling than endocervical specimens in women. To our knowledge, reports on *C trachomatis* detection in vaginal swab specimens using an EIA test are very rare. Thus, in the present study we evaluated the performance of a new improved EIA kit in the detection of *C trachomatis* in vaginal swab and endocervical swab specimens from commercial sex workers, compared with those of a conventional EIA test and a PCR assay.

Materials and methods

STUDY POPULATION

In this study, specimens were obtained from a high risk group of 163 female commercial sex workers who visited an STD clinic in Fukuoka, Japan, from July to October 1997 in order to undergo screening for major STDs, including chlamydial infection, gonorrhoea, and HIV-1 infection. Approximately 90% of commercial sex workers were asymptomatic and voluntarily sought STD check ups. All the women engaged

Table 1 Results of the detection of *C trachomatis* in endocervical and vaginal specimens from commercial sex workers by IDEIA, IDEIA PCE, and PCR

No of specimens	Endocervical			Vaginal			Patient status (no of patients)	
	IDEIA	IDEIA PCE	PCR	IDEIA	IDEIA PCE	PCR	Infected	Not infected
127	—	—	—	—	—	—	0	127
24	+	+	+	+	+	+	24	0
3	+	+	+	—	+	+	3	0
2	—	+	+	—	+	+	2	0
1 ^a	—	+	+	—	—	—	1	0
2 ^b	—	—	+	—	—	—	2	0
1 ^c	—	—	—	+	+	—	0	1
2 ^d	—	—	—	—	+	+	2	0
1 ^e	—	—	—	—	—	+	1	0
Total 163	27 ^f	30	32	25	32	32	35	128

^a, ^b, ^d, ^e All six were positive in the confirming test.

^c This was negative in the confirming test.

^f Numbers of confirmed positive cases by each assay.

in oral and vaginal sex with their clients. The women were aged between 20 and 39 years.

SAMPLE COLLECTION

Vaginal and endocervical specimens were obtained by a clinician from each woman using four different swabs. Initially, vaginal specimens were collected by placing two Dacron tipped swabs 4–6 cm into the posterior vaginal fornix.⁷ The two vaginal swabs were collected simultaneously. The swabs were rotated several times before withdrawal. Of the two vaginal swabs, one was placed into an Amplicor transport tube (Roche Molecular Systems, Branchburg, NJ, USA) and the other was placed into an EIA transport tube for IDEIA and IDEIA PCE (Dako Ltd, Ely, Cambs). Two endocervical specimens were then obtained with a speculum by inserting a swab into the endocervix. Before sampling the endocervix was cleaned with a swab to remove excess mucus. The swab was rotated several times before withdrawal. The first swab was placed into an Amplicor transport tube (Roche Molecular Systems) and the second one was placed into an EIA transport tube (Dako Ltd).

ENZYME IMMUNOASSAY

EIA specimens were processed and measured by the IDEIA test and the IDEIA PCE test, a new improved EIA kit, according to the manufacturer's instructions, respectively. The IDEIA PCE test is a new and qualitative enzyme immunoassay for the detection of chlamydial specific lipopolysaccharide (LPS) antigens. The principle of IDEIA PCE is based on the use of dual amplification. In addition to the signal amplification system used in a conventional EIA test (IDEIA), the new technology involves the use of a polymer conjugate enhanced (PCE) system consisting of a dextran backbone to which anti-Chlamydia LPS monoclonal antibody molecules are bound. Alkaline phosphatase is also bound to this backbone; hence, for every immune complex interaction multiple molecules of alkaline phosphatase are available to drive the signal generation in an enzyme amplified colour development system. It has been reported that the use of polymer conjugates can increase colour development approximately 40-fold compared with a conventional method.¹¹

POLYMERASE CHAIN REACTION

The swab specimens were stored at 2–8°C for up to 3 days until processed and measured with Amplicor *C trachomatis* test (Roche Molecular Systems) as described in detail elsewhere.^{3,4}

RESOLUTION OF DISCREPANCIES AND SUPPLEMENTARY TESTING

A woman was considered to be infected with *C trachomatis* if the IDEIA PCE test and PCR assay for both sites (endocervical and vaginal) gave positive results. When a discrepancy in the results among vaginal and endocervical specimens taken using the IDEIA PCE or PCR was observed, nested PCR assay with a different plasmid target region from that of the Amplicor test was performed as supplementary testing on specimens from both sites. The first PCR amplification was performed using primers CT2 and CT5 as described previously.¹² The reaction product was then amplified for a second time using primers CT7 (5'-GGATTTATCGGAAACCTTGA-3') and CT8 (5'-CTTTCAATGGAATAGCGGGT-3') with all other conditions remaining the same.¹² Amplified product (10 µl) was analysed by electrophoresis in a 2% agarose gel. If a woman was positive on at least one specimen, either endocervical or vaginal, using the supplementary testing, combined with one other positive test result (IDEIA PCE or Amplicor PCR), the woman was confirmed as being infected with *C trachomatis*. Following resolution of the discrepancies in the results, relative sensitivity and specificity, confidence intervals, and predictive values for each type of specimen were calculated.

Results

The results obtained using IDEIA PCE were compared with those obtained by IDEIA and PCR (Table 1). Of 163 women tested, 127 were negative and 24 were positive by IDEIA, IDEIA PCE, and PCR assay of two sample types. Specimens from 12 women demonstrated discrepancies among the results from either the assay procedures or sample sites. Of the 12 women, five were positive according to IDEIA PCE and PCR assay on both the endocervical and vaginal specimens, although these five women demonstrated discrepancies in the results when IDEIA was used on of vagi-

Table 2 Performance of IDEIA, IDEIA PCE, and PCR for the detection of *C trachomatis* in endocervical and vaginal swab specimens from commercial sex workers

Procedure/sample source	Prevalence (%)	Relative sensitivity (%)	Relative specificity (%)	Positive predictive value (%)	Negative predictive value (%)
IDEIA					
Endocervical	16.6 (27/163)	77.1 (27/35) (59.87–89.58%)	100 (128/128) (97.16–100%)	100 (27/27)	94.1 (128/136)
95% confidence interval					
Vaginal	15.3 (25/163)	68.6 (24/35) (50.72–83.12%)	99.2 (127/128) (95.73–99.98%)	96 (24/25)	92 (127/138)
95% confidence interval					
IDEIA PCE					
Endocervical	18.4 (30/163)	85.7 (30/35) (69.75–95.19%)	100 (128/128) (97.16–100%)	100 (30/30)	96.2 (128/133)
95% confidence interval					
Vaginal	19.6 (32/163)	88.6 (31/35) (73.27–96.80%)	99.2 (127/128) (95.73–99.98%)	96.9 (31/32)	96.9 (127/131)
95% confidence interval					
PCR					
Endocervical	19.6 (32/163)	91.4 (32/35) (76.95–98.19%)	100 (128/128) (97.16–100%)	100 (32/32)	97.7 (128/131)
95% confidence interval					
Vaginal	19.6 (32/163)	91.4 (32/35) (76.95–98.19%)	100 (128/128) (97.16–100%)	100 (32/32)	97.7 (128/131)
95% confidence interval					

nal and/or endocervical specimens. These five women were determined as being positive for *C trachomatis* infection. Of the remaining seven women, six were confirmed as being positive using nested PCR of the endocervical and/or vaginal specimens, and one was negative according to the nested PCR of both the endocervical and vaginal specimens. This woman was also negative according to repeat PCR of diluted specimens. Finally, of the 163 women tested, 35 were found to be infected, while 128 were found to be uninfected.

The relative sensitivity and specificity, 95% confidence intervals, and predictive values were then calculated according to these results (Table 2). The relative sensitivities of IDEIA PCE, IDEIA, and PCR on endocervical specimens were 85.7%, 77.1%, and 91.4%, respectively. Moreover, the relative sensitivities of IDEIA PCE, IDEIA, and PCR on vaginal specimens were 88.6%, 68.6%, and 91.4%, respectively. The relative specificities of these three tests were nearly 100% in the two types of specimen. These results suggest that IDEIA PCE test shows higher sensitivity than IDEIA in both endocervical and vaginal specimens, and that the sensitivity of IDEIA PCE was comparable with PCR assay in the two types of specimens. Moreover, there were no significant differences in the sensitivities of IDEIA PCE test and PCR assay between endocervical and vaginal specimens. However, the number of positive samples tested were low and this is reflected in the 95% confidence limits presented (Table 2).

Discussion

In Japan, commercial PCR or LCR assay kits are available as routine tests for the detection of *C trachomatis*. However, these DNA amplification tests are extremely costly,¹³ if used frequently for screening of *C trachomatis* among many commercial sex workers who often visit our STD clinic in the course of a year. Furthermore, these tests require specialised facilities to reduce Amplicor contaminants. On the other hand, although EIA is less sensitive than PCR or LCR, this procedure has several advantages such as low cost and does not require sophisticated laboratory facilities. More recently, IDEIA PCE, a new and qualitative EIA kit, has been developed by Dako Ltd. The performance of the IDEIA

PCE test has been improved with 2.5-fold to 5-fold higher sensitivity in the detection of viable cells of laboratory propagated *C trachomatis*, compared with a conventional EIA test (IDEIA).¹⁴ In the present study, we compared the performance of IDEIA PCE with those of the IDEIA test and the commercially available PCR assay in the clinical samples.

Currently, several studies have demonstrated that analysis of vaginal specimens or urine specimens using DNA amplification method is a useful alternative to endocervical specimens obtained for chlamydia diagnosis in women.^{7–10 15 16} However, in our previous study of women comparing LCR on urine specimens with that on endocervical specimens, urine specimens were positive in only 35 of 48 (73%) infected women.¹⁷ The reason for lower sensitivity of urine from women is that most women are infected with *C trachomatis* at the endocervix, a site remote from the urethra. Therefore, female urine detection may not adequately identify endocervical infection. Moreover, handling and laboratory processing of urine specimens are more difficult compared with endocervical or vaginal swab specimens. Thus, in the present study, we evaluated clinical significance of the vaginal specimens using a new EIA kit.

The results in this study demonstrated that the sensitivities of IDEIA PCE on vaginal and endocervical swab specimens (vaginal swab, 89%; endocervical swab, 86%) were greater than those of IDEIA (vaginal swab, 69%; endocervical swab, 77%), and were comparable with PCR assay (vaginal swab, 91%; endocervical swab, 91%). Interestingly, there were no significant differences in the sensitivities and specificities of IDEIA PCE and PCR between vaginal and endocervical swab specimens. Although IDEIA PCE test may be theoretically less sensitive than PCR, the performance of the new EIA test was the same as that of PCR in clinical samples. This is probably because of the presence of a high number of *C trachomatis* infected cells in clinical specimens, which the IDEIA PCE test is able to detect. In a previous reported study on a high prevalence population¹⁸ the IDEIA Chlamydia test was shown to have equivalent sensitivity to LCR when applied to urine from men with urethritis. The vaginal swab using IDEIA PCE appears to be an acceptable, sensitive, and less

invasive approach for the detection of *C trachomatis* in women. However, in this study no comparison was made of the adequacy of the sample collected (for example, prevalence of columnar epithelial cells). Although the specimen collection procedure was considered to be appropriate, it is possible that some of the discrepant results obtained between sample types were due to suboptimal sample collection. The population size tested and number of positive samples are not sufficient to predict accurately the actual sensitivity of the test, and this is reflected in 95% confidence intervals presented. The data indicate the relative sensitivity of the tests used and the use of vaginal swabs as a potential alternative to endocervical swabs. The discrepant analysis procedure used was only applied to the discrepant samples and not the whole population tested and this may have introduced some bias with the data analysis.¹⁹ A large study is required to assess the true clinical performance and value of vaginal swabs as an alternative to endocervical swabs.

The prevalence rate of *C trachomatis* in commercial sex workers tested was approximately 20%. This prevalence rate among the women seems to be significantly higher than that in the Japanese general female population (approximately 5%).²⁰ In our city, female commercial sex workers are a major reservoir of STDs. To prevent the wide spread of *C trachomatis* infection to the general population, continuous close monitoring of *C trachomatis* infection among commercial sex workers is necessary. In this regard, vaginal swabs using the IDEIA PCE test give us a convenient tool in the screening of *C trachomatis* among commercial sex workers. Although the sensitivity of the IDEIA PCE Chlamydia is greater than IDEIA Chlamydia, the cost per test is similar and much lower than PCR. The use of IDEIA PCE Chlamydia applied to vaginal swabs offers the potential for cost effective reliable screening of high prevalence female populations. Although the population tested was mainly asymptomatic, the prevalence was high because of the occupation of the population tested. The relative sensitivity and specificity obtained with the tests used and samples tested may not be applicable to lower prevalence populations—for example, family planning clinic, because the carriage of *C trachomatis* will be lower.

Recent publications have shown that DNA amplification testing for chlamydia with patient obtained vaginal swabs is as sensitive as endocervical testing.^{8–10} Patient obtained vaginal swabs seem to be a more suitable specimen for the screening of *C trachomatis* than clinician obtained endocervical or vaginal specimens.

However, we did not evaluate the sensitivity of IDEIA PCE on patient obtained vaginal swab specimens. Therefore, we will choose patient obtained vaginal swabs for the detection of *C trachomatis* in our next project.

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